Scatter Factor/Hepatocyte Growth Factor Protects against Cytotoxic Death in Human Glioblastoma via Phosphatidylinositol 3-Kinase- and AKT-dependent Pathways

Daniel C. Bowers, Saijun Fan, Kevin A. Walter, Roger Abounader, Jeffery A. Williams, Eliot M. Rosen, and John Laterra

INTRODUCTION

High-grade malignant gliomas are the most common cancers of the central nervous system. Despite treatment with surgical resection, radiation therapy, and chemotherapy, the mean overall survival is <1.5 years, and few patients survive for >3 years. A common reason for treatment failure is their innate resistance to radiation and chemotherapy. The mechanisms by which tumor cells survive death induced by these cytotoxic treatment modalities are becoming increasingly defined and involve growth/angiogenic factors, cytokines, and a diverse array of parallel and overlapping signaling pathways involved in the regulation of DNA repair and apoptosis. Identifying and ultimately targeting the mechanisms by which gliomas resist cytotoxic agents is likely to have a substantial impact on future treatment strategies.

SF, also known as HGF, is a multifunctional Mr 90,000 heterodimeric growth factor, motility factor, and morphogen that, through its transmembrane tyrosine kinase receptor, the product of the c-met proto-oncogene, regulates developmental, regenerative, and neoplastic processes (1–5). SF/HGF and c-met are aberrantly expressed by a variety of cancers, including malignant gliomas, renal cell carcinomas, breast carcinomas, and sarcomas (6–10). In cancer cells, excessive c-met activation has been shown to induce transformation, tumor growth, tumor cell motility, invasion of extracellular matrices, and angiogenesis (6, 7, 11–14). SF/HGF: c-met signaling has also been found recently to function as a survival pathway and an inhibitor of apoptosis. SF/HGF protects carcinoma cells from apoptosis induced by DNA-damaging agents (15, 16), and expression of constitutively active c-met inhibits hepatocyte apoptosis (17). The antiapoptotic proteins BAG-1 (18) and Bcl-X, (16) have been implicated in the cytoprotective mechanisms of SF/HGF in carcinoma cell lines.

Human malignant gliomas frequently express both SF/HGF and c-met (11, 19, 20), which can establish an autocrine loop of biological significance (21). Glioma c-met expression correlates with glioma grade (20), and an analysis of human tumor specimens showed that malignant gliomas have a 7-fold higher SF/HGF content than low-grade gliomas (22). SF/HGF has been shown to promote dissemination of glioma cells, glioma cell proliferation, and glioma-associated angiogenesis (8, 14, 19). Inhibiting the expression of endogenous SF/HGF or c-met in human glioblastoma cells can reverse their malignant phenotype in vitro and in vivo (21). A role for SF/HGF:c-met signaling in the chemoresistance and radioresistance of malignant gliomas has not been established.

This study uses a human malignant glioma and a rat gliosarcoma cell line to show that SF/HGF can inhibit glioma cytotoxicity and apoptosis induced by either radiation or chemotherapeutic agents in vitro and in vivo. Inhibition of c-met gene expression by U1/ribozyme gene transfer demonstrated that the cytoprotective action of SF/HGF is c-met dependent. Furthermore, we show that SF/HGF activates the antiapoptotic factor Akt and that the cytoprotective action of SF/HGF is dependent, in part, upon Akt as well as on phosphatidylinositol 3-kinase upstream from Akt activation. To our knowledge, this is the first description of an endogenous cytokine/growth factor that functions as a cytoprotective agent by acting directly on glioma cells.

MATERIALS AND METHODS

Cell Culture. Human U-373 MG (U-373) glioma cells were originally obtained from American Type Culture Collection (Rockville, MD). U-373 cell lines stably transfected with chimeric U1 small nuclear RNA/ribozyme constructs designed to inhibit endogenous c-met expression (designated U373-
SF/HGF protects gliomas from cell death

Sorbace units of samples of control cells. DMSO were added to each well. Absorbance at 570 nm was measured by exposure. Data indicate means; as the mean absorbance of cells exposed to cytotoxic agents divided by plates for MTT cytotoxicity assays.

Expression Vectors and Transient Cell Transfections. The Akt expression vectors (23) were kindly provided by Dr. Michael J. Quon (National Heart, Lung, and Blood Institute, NIH, Bethesda, MD). These include mammalian expression vectors encoding wild-type Akt, constitutively active Akt (Akt-myr), a kinase-inactivated, dominant-negative Akt (K179A), and the empty expression vector pCIS2. Transient transfection was performed by incubating proliferating cells at ~50% of confluence overnight with 10 μg of plasmid DNA per 100 mm dish in the presence of Lipofectamine (Life Technologies, Inc.). Cells were then rinsed and incubated with complete medium for an additional 24 h prior to trypsinization and plating into 96-well plates for MTT cytotoxicity assays.

MTT Cytotoxicity Assay. U-373 cells were plated at 5 × 10^4 cells/well in groups of 8–10 in 96-well culture plates, in medium containing recombinant human SF/HGF (a kind gift of Genentech, Inc., S. San Francisco, CA) at the indicated concentrations. PBS only was used as control. After 48 h, cells were exposed to the indicated concentrations of either Adriamycin (Sigma Corp., St. Louis, MO) for 2 h, paclitaxel (Sigma) for 2 h, cisplatin (Sigma) for 2 h, camptothecin (Sigma) for 48 h, or to ionizing radiation (radiation source = 32P Cesium GammaCell Irradiator; Atomic Energy of Canada, Ltd., Mississauga, Ontario, Canada) in a single fraction. After 48 additional h from the time of starting the exposure to each cytotoxic agent, MTT (1 mg/ml; Sigma) was added to each well and incubated for 2 h at 37°C. The medium was then removed, and 100 μl of DMSO were added to each well. Absorbance at 570 nm was measured by a microculture plate reader (Dynatech; Alexandria, VA). Cell survival was calculated by absorbance units of samples of treated cells/mean absorbance units of samples of control cells.

In Vivo Cytotoxicity. 9L-SF or control 9L-neo (10^3 cells/animal) were implanted by stereotaxic injection into the caudate-putamen of male Fischer 344 rats (weight, 200–250 g) as described previously (14). Seven days after injection of tumor cells, restrained and air-breathing animals were positioned a fixed distance from a calibrated linear accelerator (Varian 2300, Palo Alto, CA). Using 10 MeV energy, animals received 2000 cGy cranial irradiation via vertex port alignment in a single fraction as described previously (24). Animals were anesthetized and sacrificed 18 h later by intracardiac perfusion with 1% SDS and 2.5 mg/ml RNase A for 2 h. The pellets were then suspended in electrophoresis loading buffer and electrophoretically separated in U373-Met-KD cell lines, total cell extracts were obtained from subconfluent cell monolayers using RIPA buffer containing protease inhibitors and concentrated as described above. Western immunoblot analysis of samples was performed as described below.

Western Blot Analysis. SDS-PAGE and immunoblotting were performed according to the methods of Towbin et al. (26) with modifications. Cell extracts containing 20 μg of protein were diluted in Laemmli buffer containing β-mercaptoethanol and heated to 100°C for 2 min. The proteins were separated by SDS-PAGE at 50 mA for 20 min. Proteins were then electrophoretically transferred to Hybond ECL nitrocellulose membranes (Amersham Life Science, Buckinghamshire, England). The membranes were incubated with 5% nonfat dried milk in Tris-buffered saline (100 mM Tris-HCl, 0.9% NaCl, pH 7.4) for 1 h and then incubated with a 1:1000 dilution of either rabbit polyclonal anti-Akt or anti-phosphorylated Akt (Ser473) IgG (New England Biolabs, Beverly, MA) overnight at 4°C. After washing, the membranes were incubated for 1 h with a 1:1000 dilution of horseradish peroxidase-conjugated goat antirabbit IgG (Jackson ImmunoResearch, West Grove, PA). The membrane-bound antibodies were then visualized using an ECL Western Blotting Detection kit (Amersham) on ECL Film. The films were digitized and quantitatively analyzed by densitometry (Molecular Dynamics, Sunnyvale, CA).

DNA Fragmentation Assay. Chromosomal DNA fragmentation in response to cytotoxic agents was determined by agarose gel electrophoresis as described previously (16, 27). Control cells or cells treated with Adriamycin (15 μM) in the presence or absence of SF/HGF were lysed in a solution containing 50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, and 1% NP40. The supernatants were incubated with 1% SDS and 2.5 mg/ml RNase A for 2 h at 37°C and then incubated overnight with 2.5 mg/ml proteinase K at 56°C. DNA was precipitated with ammonium acetate and 95% ethanol. DNA was resuspended in electrophoresis loading buffer and electrophoretically separated in agarose gels.
1.5% agrose containing 0.1 mg/ml ethidium bromide to visualize “DNA ladders” that are characteristic of apoptosis.

**DNA Filter Elution Assays.** Subconfluent cell monolayers were incubated with medium containing [3H]thymidine (0.02 μCi/ml) for 32 h, followed by incubation in isotope-free medium for an additional 2 h. Cells were then incubated with SF/HGF and either Adriamycin (15 μM) or camptothecin (20 μM) as described above. Cells were then incubated in complete medium for 24 h and suspended by incubation with trypsin/EDTA. Equal numbers of cells (2 × 10^6) were applied to nonproteinizing polycarbonate membranes, lysed, and subjected to alkaline elution for detection of single-strand DNA breaks as described previously (28). Radioactivity was quantified by scintillation spectrophotometry, and the fraction of eluted DNA was calculated according to the formula: dpm eluted/(dpm filter-bound + dpm total lysate).

**RESULTS**

**SF/HGF Protects Glioblastoma Cells from DNA-damaging Agents in Vitro.** A microtiter spectrophotometric assay that measures the capacity of mitochondria to convert tetrazolium bromide (MTT) to formazen was used to quantify the relative survival of U-373 glioblastoma cells exposed to cytotoxic agents in the presence or absence of SF/HGF. Pretreatment of cells for 48 h with purified recombinant SF/HGF significantly increased the fraction of U-373 cells surviving exposure to either ionizing gamma radiation, the DNA cross-linking agent cisplatin (100 μM), or the topoisomerase I inhibitor, camptothecin (20 μM). The cytoprotective effects of SF/HGF were concentration dependent with statistically significant increases in cell survival occurring at SF/HGF concentrations ≥1.0 ng/ml. SF/HGF (3.0 ng/ml) increased the fraction of cells that survived exposure to either 5.0 Gy gamma irradiation, cisplatin, or camptothecin by 33% (P < 0.05), 77% (P < 0.01), and 85% (P < 0.001), respectively (Fig. 1). Preincubation of cells with SF/HGF was required for cytoprotection because treating cells with SF/HGF only during their exposure to DNA-damaging agents had no effect on cell survival (not shown).

**SF/HGF Reduces Glioblastoma Cell Apoptosis Induced by DNA-damaging Agents in Vitro and in Vivo.** The effect of SF/HGF on the fraction of cells undergoing apoptosis in response to cytotoxic agents in vitro was examined by TUNEL immunocytochemistry. In the absence of SF/HGF, 3.3% of U-373 cells became TUNEL positive after 5.0 Gy gamma irradiation; 6.8% of cells became TUNEL positive after exposure to cisplatin (30 μM), and 2.9% became TUNEL positive after exposure to camptothecin (20 μM). Fewer than 0.1% of cells were TUNEL positive in control cultures exposed to buffer only under identical conditions. SF/HGF reduced the fraction of apoptotic nuclei after exposure to either chemotherapy or ionizing gamma radiation. Pretreatment of cells with 3.0 ng/ml recombinant human SF/HGF resulted in a 38% reduction in the fraction of TUNEL-positive cells exposed to radiation (P < 0.001), a 35% reduction of TUNEL-positive cells exposed to cisplatin (P < 0.001), and a 79% reduction of TUNEL-positive cells exposed to camptothecin (P < 0.001; Fig. 2, A and B).

Human SF/HGF gene transfer was used to examine the effect of SF/HGF production by glioma cells on radiation-induced apoptosis in vivo. We chose the 9L gliosarcoma model for these experiments because 9L glioma cells generate more rapidly growing and reproducible tumors than U-373 cells. However, like U-373 cells, 9L cells normally express the SF/HGF receptor c-met but lack detectable SF/HGF expression (13). Stable control-transfected SF/HGF-negative 9L cells (9L-neo) and 9L cells engineered to secrete human SF/HGF (9L-SF) were established and characterized as reported previously (13). 9L-neo and 9L-SF cells were stereotactically implanted to caudate-putamen of syngeneic Fischer 344 rats to produce control SF/HGF-
negative and SF/HGF-positive brain tumors, respectively (14). Seven days later, animals bearing established tumors were exposed to 2.0 Gy cranial irradiation in a single vertex fraction, or they were sham-irradiated as control. Animals were perfusion fixed 24 h later, and tumor sections were analyzed for tumor cell apoptosis by TUNEL immunohistochemistry. The percentage of TUNEL-positive cells found in unirradiated tumors was <0.025% and did not differ between control 9L-neo and 9L-SF tumors. Radiation increased the percentage of TUNEL-positive cells in the 9L-neo tumors by >25-fold to 0.67%. In contrast, the percentage of TUNEL-positive cells in the irradiated 9L-SF tumors increased to only 0.23%, approximately one-third of that seen in the irradiated control tumors (P < 0.001; Fig. 2C).

Anti-c-met Ribozyme Inhibits Cytoprotection by SF/HGF. To determine the role of the c-met receptor in the cytoprotective action of SF/HGF, we examined the cytoprotective effects of SF/HGF in control-transfected U-373 cells (U373-CT) and in U-373 cells stably transfected with a chimeric U1/ribozyme previously shown to specifically inhibit (i.e., knock-down) human c-met expression (designated U373-Met-KD; Ref. 21). Extracts from U373-Met-KD cell lines were found to contain approximately 5–11% of c-met mRNA and approximately 2–23% of c-met protein when compared with U373-CT cells, as determined by Northern hybridization and immunoblotting, respectively (Fig. 3; Ref. 21).

The effect of SF/HGF on the response of U373-CT cells and U373-Met-KD cells to DNA-damaging agents was examined using three distinct end points of cell injury and DNA damage. As expected based on the results described above, preincubation of U373-CT cells with SF/HGF increased the fraction of cells surviving incubation with Adriamycin, cisplatin, and Taxol as determined by MTT assay by 114, 68, and 76%, respectively. In contrast, SF/HGF enhanced the survival of U373-Met-KD cell lines after their exposure to the same cytotoxic agents by only 21, 18, and 42%, respectively (Fig. 4A). Thus, anti-c-met ribozyme reduced SF/HGF-mediated cytoprotection by approximately 45–80% (P < 0.001). Likewise, SF/HGF substantially inhibited Adriamycin-induced DNA laddering in U373-CT cells but had little effect on this marker of apoptosis in U373-Met-KD cells (Fig. 4B).

Fig. 3. Inhibition of c-met expression in U-373 cells by U1/ribozymes. Wild-type U-373 cells were stably transfected with control plasmid or with plasmid bearing a transgene coding for anti-c-met U1/ribozyme as described in “Materials and Methods.” A, total cellular RNA derived from two control-transfected cell lines (CT) and from three U1/ribozyme-transfected cell lines (KD) was analyzed by Northern hybridization using c-met and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probes. B, total cellular protein obtained from the same control-transfected cell lines and U1/ribozyme-transfected cell lines was analyzed by immunoblot analysis (10 μg total protein/lane) using anti-c-met antibodies. Specific antibody binding to membrane was detected by chemiluminescence. Substantial knock-down (KD) of c-met expression resulted from U1/ribozyme transfection as evidenced by reductions in c-met RNA relative to GAPDH and by reductions in immunoreactive c-met protein.

Fig. 4. C-met knock-down by U1/ribozyme inhibits SF/HGF cytoprotection. Stable control-transfected (CT) and anti-c-met U1/ribozyme-transfected (MET-KD) U-373 cell lines were established and characterized as described in “Materials and Methods” and in Fig. 3. A, CT and MET-KD cell lines were treated with PBS or SF/HGF for 48 h. Cells were then exposed to either Adriamycin (ADR, 15 μM), cisplatin (CDDP, 20 μM), or Taxol (20 μM), and the fraction of cells that survived exposure to each cytotoxic agent was quantified by MTT assay as described in “Materials and Methods.” The percentage of increase in cell survival attributed to SF/HGF treatment for each cell line was determined according to the formula: (fractional survival of SF/HGF-treated cells – fractional survival of PBS-treated cells) × 100. Data represent results from two control (CT) cell lines and three MET-KD cell lines (n = 10). Data indicate means; bars, SE; P < 0.001 comparing CT to MET-KD cell lines for each cytotoxic agent tested. B, U373-CT and U373-met-KD cell lines were incubated with SF/HGF or buffer only for 48 h and then treated with Adriamycin (ADR, 15 μM) or buffer only for an additional 1 h. Cells were then harvested, and cellular DNA was isolated and electrophoretically separated as described in “Materials and Methods.” SF/HGF substantially reduced the extent of Adriamycin-induced DNA fragmentation (e.g., laddering) in U373-CT cells but had little effect in met-KD cells. The figure is representative of results obtained with two control and three met-KD cell lines and was repeated at least twice. Subconfluent proliferating U373-CT (CT) and U373-Met-KD (MET-KD) cells were treated with PBS or with SF/HGF for 48 h, exposed to Adriamycin (ADR, 15 μM) or to camptothecin (CPT, 25 μM) for 2 h, and then incubated for an additional 24 h in complete medium. The number of DNA single strand breaks were then determined by DNA elution assay as described in “Materials and Methods.” SF/HGF treatment significantly reduced the number of Adriamycin-induced DNA stand breaks in U373-CT cells but had no effect in met-KD cells. Data represent results from two CT and three met-KD cell lines (n = 10). Data are means; bars, SE; * P < 0.001 comparing –SF/HGF to +SF/HGF.
Ribozyme inhibition of c-met expression completely reversed the ability of SF/HGF to protect glioblastoma cells from chemotherapy-induced DNA single-strand breakage as determined by DNA filter elution assay (Fig. 4C).

**SF/HGF Cytoprotection Is PI3-Kinase Dependent and Akt Dependent.** PI3-Kinase-dependent activation of Akt has emerged recently as an important mechanism by which malignant cells gain resistance to cytotoxic agents (29, 30). We asked whether SF/HGF increases Akt activation in glioblastoma cells using polyclonal antibodies that specifically recognize either the activated form of Akt (phosphorylated at serine residue 473) or total Akt. Incubating U-373 cells with SF/HGF for 2–48 h resulted in an ~5-fold increase in the phosphorylated form of c-Akt protein relative to total cellular Akt (Fig. 5). Incubation of U-373 cells with either wortmannin (50 nM) or LY249002 (10 μM), distinct selective inhibitors of PI3-kinase, completely inhibited the activation of Akt by SF/HGF (Fig. 5). In contrast, Akt activation by SF/HGF was minimally effected by treatment with PD98059 (30 μM), a mitogen-activated protein kinase kinase inhibitor, or with GF109302X (1 μM), a protein kinase C inhibitor (Fig. 5).

We next asked whether inhibition of PI3-kinase, under conditions that block SF/HGF-mediated Akt activation, altered the cytoprotective action of SF/HGF in U-373 cells. Cells were pretreated with SF/HGF in the presence or absence of either wortmannin or LY294002 and then exposed to camptothecin as described earlier. At the concentrations used in these experiments, neither wortmannin nor LY294002 altered the basal viability of U373 cells. LY294002 and wortmannin inhibited the capacity of SF/HGF to increase cell survival by approximately 80 and 50%, respectively (Fig. 6A). Neither PD98059, the mitogen-activated protein kinase kinase inhibitor, nor GF109302X, the protein kinase C inhibitor, altered SF/HGF-mediated cytoprotection. To directly examine the role of c-Akt in SF/HGF cytoprotection, U373 cells were transiently transfected with either a dominant-negative kinase-inactivated Akt (DNAkt), constitutively activated Akt (Akt-myr), wild-type Akt, or empty expression vector as control (all kindly provided by Dr. Michael Quon, NIH, Bethesda, MD) prior to treatment with SF/HGF and/or camptothecin (Fig. 6B). Expression of wild-type Akt reduced the cytotoxicity of camptothecin by ~50%, but these cells remained sensitive to the cytoprotective action of SF/HGF. Expression of constitutively active Akt completely blocked the cytotoxicity of camptothecin. Expression of dominant-negative Akt had no significant effect on the cytotoxicity of camptothecin but inhibited the cytoprotective action of SF/HGF by ~50%.

**DISCUSSION**

Tumors gain resistance to cytotoxic agents as a result of several different molecular mechanisms. These include enhanced DNA repair, multidrug resistance associated protein-mediated exportation of cytotoxic drugs, alterations in intracellular glutathione and metallothioneine concentrations, increased expression of aldehyde dehydrogenase and O⁵-alkylguanine-DNA-alkyltransferase, and structural alteration of microtubules (31, 32). Furthermore, mutations in the p53 tumor suppressor gene and alterations in the levels of topoisomerase may also contribute to the induction of chemotherapy and radiation therapy-resistant phenotypes (33). Finally, expression of members of the Bcl family of apoptosis regulating proteins and the interleukin-1β-converting enzyme-like family of cysteine proteases (also termed caspases) influence the susceptibility of tumors to apoptosis (34). Growth factors, such as acidic and basic fibroblast growth factor, insulin-like growth factor-I, and cytokines are able to protect certain tumor cell lines from cytotoxic cell death (35, 36). Better understanding of these mechanisms will lead to new strategies to inhibit tumor cell resistance to existing cytotoxic therapies.

We show in this report that the multifunctional growth factor and angiogenic factor SF/HGF, which has recently been implicated in glioma malignancy, decreases cell death, apoptosis, and DNA strand...
inhibited by assay. The cytoprotective action of SF/HGF was between G0-G1 and S-phase (42, 43). Camptothecin inhibits topoisomerase I in DNA, depressing DNA synthesis and causing a transient block in cell cycle (44). Cisplatin forms covalent adducts between adjacent guanosine residues in DNA, thereby stabilizing tubulin and blocking cell cycle progression through mitosis (44). Adriamycin has multiple mechanisms of action that include direct intercalation and covalent binding to DNA and inhibition of topoisomerases. That SF/HGF is cytoprotective against such an array of agents suggests a mechanism of action involving pathways such as DNA repair and antiapoptosis that are fundamental to cell evasion of death signals. We demonstrate in U-373 human malignant glioma cells that SF/HGF activates the PI3-kinase/Akt pathway that mediates growth factor-induced cell survival and suppresses apoptotic death induced by various stimuli, including growth factor withdrawal, cell cycle discordance, loss of cell adhesion, and DNA damage in many cell types. In vitro cytoprotection was completely inhibited by two distinct PI3-kinase inhibitors. PI3-kinase activates a number of cell signaling pathways with cytoprotective functions. Akt is activated by PI3-kinase through phospholipid binding and phosphorylation at Thr308 and Ser473 (45). We show that SF/HGF stimulates a substantial increase in active phospho-Akt, and Akt-dependent pathway that is presently being explored.

Importantly, we also demonstrate that transgenic SF/HGF expression in intracranial rat 9L gliomas inhibits their apoptotic response to gamma irradiation in vivo. These findings are consistent with the cytoprotective action of SF/HGF reported recently in other systemic tumor cell lines and in nonneoplastic cells. Recently, we showed that SF/HGF blocks the induction of cell death and apoptosis by various DNA-damaging agents, including chemotherapy, ionizing radiation, and UV in human breast cancer cells and mouse mammary tumor cells (16). Renal epithelial cells transfected to produce SF/HGF are more resistant to anoikis (47). Our finding that SF/HGF inhibits the ability of Adriamycin to down-regulate the antiapoptosis protein, Bcl-XL, in MDA-MB-453 carcinoma cells (16). Furthermore, Bardelli et al. (18) reported binding of the antiapoptotic protein, BAG-1, with the SF/HGF receptor c-met, and overexpression of BAG-1 enhances hepatocyte progenitor cell survival from apoptosis. In addition, activation of Akt by SF/HGF could result in the phosphorylation of pro-caspase-9 at Ser196 and thereby promote cell survival by inhibiting its proteolytic activity (47). Our finding that Akt-dependent Akt only partially reverses SF/HGF cytoprotection suggests a potential role for a PI3-kinase-dependent but Akt-independent pathway that is presently being explored.

In conclusion, we establish in this report that SF/HGF functions as a glioma cell survival factor both in vitro and in vivo. A mechanism involving PI3-kinase and Akt is proposed based upon the in vitro analysis of the U-373 human malignant glioma cell line. Strategies developed recently to inhibit SF/HGF:c-met signaling by targeting the cysteine rich domain of the c-met receptor are being explored in our laboratory.
gene expression (21) or c-met receptor function (48) may be useful in enhancing the therapeutic response of malignant gliomas to existing conventional cytotoxic modalities.

ACKNOWLEDGMENTS

We thank Angela T. Williams for help with manuscript preparation.

REFERENCES


Scatter Factor/Hepatocyte Growth Factor Protects against Cytotoxic Death in Human Glioblastoma via Phosphatidylinositol 3-Kinase- and AKT-dependent Pathways


*Cancer Res* 2000;60:4277-4283.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/15/4277

Cited articles
This article cites 47 articles, 18 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/15/4277.full#ref-list-1

Citing articles
This article has been cited by 42 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/60/15/4277.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.